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# ALUMINUM HYDROXIDE VACCINE AGAINST ANTHRAX

by

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After obtaining the anthrax vaccines of L. Pasteur and L. S. Tsenkovski, the veterinary microbiologists had success in starting to solve questions of obtaining inoculative means for the immunology of infectious diseases.

In 1891, I. N. Lange, using the Pasteur and Tsenkovski method, produced an anti-anthrax vaccine, which was widely used during a number of years.

F. A. Terentiev (1934) attempted to improv. Tsenkovski's second vaccine by adding it to a solution of saponin. The saponin vaccine (possessing immunological characteristics, however, appeared non-standard in its application. This was due to the fact that the saponin used for the production of the vaccine, possessed a strong toxic quality and caused the destruction of spores contained in the vaccine. In 1947, the saponin vaccine was removed from (utilization) the market.

E. Stamatin (1934) in studying the morphology and biology of virulent anthrax cultures (grown on coagulated defibrinogenous or citrinous horse blood), discovered the possibility of obtaining weakly virulent, edema forming, non-spore forming strains. Stamatin produced a vaccine from such a strain and reported it for the vaccination of animals against anthrax.

In 1942 M. M. Ginsburg proposed an anthrax vaccine 'STI' for the immunization of animals. The vaccine represented a weakly virulent culture of non-capsuling microbes and appears as a high-co-immunological preparation, recommended in wide practice. The 'STI' vaccine, a preparation, used for a single injection, facilitates considerably the task of the veterinary workers.

During the last 15-20 years, the foreign scientists suggested a number of anthrax vaccines, possessing effective qualities. This was reported in detail in the article by S. Y. Kolesov in the periodical 'Veterinary Science' Volume 1, 1957.

With the help of the 'STI' vaccines and Tsenkovski's product, the practical veterinary workers conducted a successful fight against anthrax. However, at times these vaccines caused considerable post-vaccination complication on animals with a reduced resistance in their organisms. Hence, the necessity remained to continue the research and find a less harmful vaccine suitable for the creation of an immunity against anthrax. For this purpose studies were conducted for a number of years in the laboratory - controlling anthrax preparations of the Governmental Science - Control Institute for Veterinary Preparations ('VETI') USSR.

One of the authors of this paper (S. G. Kolesov) (1944-1949), while investigating a virulent strain 'Myes-2', isolated from the carcass of a pig, a non-capsuling, weakly virulent variation M (8h)-15. This variation, when tested on the laboratory animals, proved to be harmless for rabbits and weakly virulent for guinea pigs and white mice.

In 1950-1951 Messrs. S. G. Kolesov and Y. F. Borisovitch, by way of 'directed selection' obtained from the virulent anthrax strains another 4 weakly virulent alternates No. 1260-31; 94; K3-99 and K12-46.

In testing them on rabbits, these new variations appeared immunological. However, in checking them experimentally on sheep, in 1951, S. G. Kolesov and Y. F. Borisovitch ascertained that only the strain (variation) M-15 possessed highly immunological qualities. It prevented anthrax among 7 out of 8 sheep, injected 4 months prior to the controlled inoculation. Hence, investigations of this strain continued, as it was the most suitable for a new vaccine.

A checking of the harmlessness of the vaccine obtained from strain M-15 on young and grown sheep, goats, large horned cattle and horses, proved the vaccine to be harmless for the above animals. During the period of injection, the horses continued to work. As a rule, the injections did not cause any complications. The animals manifested only an insignificant local and temperature reaction.

Subsequently, the vaccine strain and the vaccines produced from it, were tested several times for their cultural and biological characteristics. It was established that the strain was resistant and maintained its original characteristics. It was a typical anthrax culture, possessing a slight residual virulence.

In 1952 a few experiments were made to investigate the possibility of raising the immunological qualities of the vaccine, by adding to it hydroxide aluminum, as an unspecific irritant and deponent. The results of the experiments established that the vaccine obtained from the strain M-15, grown on glycerin with added hydroxide of aluminum, proved more immunological, than the glycerin vaccine. After this, the vaccine was produced on a 20% solution of glycerin with an added 40% of 3% hydroxide aluminum (Table 1).

Table 1

Results of the Comparative Checkings of the Vaccine for the Immunizing Qualities,  
When Used on Rabbits

Name of Vaccine	No. of tests	No. of rabbits	Dose (c.c.)	Perished	Survived	%
Vaccine in glycerin	2	9	2	1	8	88.8
	3	10	1	7	3	30.0
	1	5	0.5	1	4	80.0
	2	15	0.3	7	8	53.3
	-	45	-	16	29	64.4
Vaccine on glycerin with added hydro-oxide aluminum (COA)	1	5	2	-	5	100.0
	2	11	1	2	9	81.8
	1	5	0.5	-	5	100.0
	2	14	0.3	6	8	57.1
	-	35	-	8	27	77.1
Control of virus	2	8	-	8	-	-

As a result of the many tests, conducted with the obtained vaccine, to determine the harmlessness on rabbits and its residual virulence on guinea pigs, it was established that the vaccine is harmless for rabbits in a dose of 190 mln spores. The test results on guinea pigs are expressed in Table 2.

The data of the table show that the new anthrax vaccine is less virulent and less reactive than the vaccine 'STI' (CTV), though almost a double dose was injected into guinea pigs. Thus, the average number of spores in 1 ml of the new vaccine amounted to 44.8 mln, whereas in the vaccine (STI) CTV = 26.1 mln.

In producing the vaccine from the strain M-15, we ascertained that there was an insufficiency in spore formation. A growing on the usual (MHA) MPA after 3 days at a raised temperature manifested 40-50% microscopically visible spores and on the 4-5th day approximately 70%.

Therefore, even during the first tests, special attention had to be given to a study of an appropriate nutrient medium in order to augment the spore formation of the strain during the production of the vaccine. As a result of this study, it was established that the best spore-formation occurred on agar, produced from a 75% 'beet' (?) fluid or from a meat hydrolyte with a content of 100-120 mg l of nitrogen and 25% 'pea extract'(?). The spore formation occurred faster on this type of medium, reaching 80-85% on the fourth day; and 90-95% on the 5th day, when examined under the microscope.

Thus, one of the complex questions, the question of spore-formation and virulence of the microbes in the shape of spores, was solved positively. This question is extremely vital, as it bears on the immunology of the preparation.

During the process of studying the hydro-oxide-aluminum vaccine, another not less vital question arose: the creation of a more adhesive medium in the vaccine; the very first tests revealed that hydro-oxide-aluminum precipitates and draws along a considerable number of spores. In order to augment the adhesiveness of the vaccine, agar was added in the quantity of 0.05-0.07-0.09 and 0.1%. With an addition of 0.09-0.1% of agar, the hydro-oxide-aluminum did not precipitate too fast, and the vaccine remained sufficiently liquid. (It may be drawn in by a syringe (needle) 05.)

The hydro-oxide-aluminum vaccine, produced in accordance with this method, was tested for its effect on guinea pigs. In the past we did not make these tests, considering the extreme sensitivity of guinea pigs to anthrax and in view of the difficulty to obtain positive results. In carrying out these tests on control guinea pigs, we utilized Tschukovski's second vaccine.

TABLE 2

Results of Checking the Vaccines for Their Virulence and Safety on Guinea Pigs

Name of Vaccine and No. of Series	No. of Tests	No. of Guinea Pigs	Dose of Vaccine	No. Reaction	Local reaction			Period of Observation	No. of Guinea Pigs	Virulence	
					degree						
					1	2	3				
Vaccine on Glycerine (series 13, 17 & 19)	4	24	0.5	1	8	9	4	1	2	22	51.0
		24	1.0	2	3	12	10	2	3	31	51.1
		58	-	3	13	21	14	6	1	53	51.4
Vaccine on Glycerine GSA (series 13, 14, 14 x 18)	3	8	0.5	-	8	-	-	-	-	8	100.0
		43	1.0	4	18	12	6	3	4	39	50.6
		8	1.5	1	2	1	2	1	2	6	75.1
Vaccine STI (CTN) series (93, 189, 14, 221, 144 and 211)		59	-	3	28	13	8	4	6	53	69.8
	4	4	0.5	1	1	2	-	-	-	4	100.0
		43	1.0	1	1	11	20	17	3	19	26
		5	1.5	-	-	-	3	1	3	3	100.0
		54	-	2	2	13	17	18	6	32	59.2

As a result it was established that hydro-oxide-aluminum vaccine may be tested against anthrax, for its immunological qualities on guinea pigs, by using a dose of 0.5 ml.

For the infection of vaccinated and control-guinea pigs, Tschukovski's second vaccine was used in a dose of 150-200 thousand virulent spores. All control guinea pigs perished within 2-3 days, while the vaccinated animals remained alive (or a minimum of 4 out of 5). Thus, we were the first ones who devised and proposed a method for the testing of anti-anthrax vaccine for its immunological characteristics (on guinea pigs).

The preparation (production) and control of hydro-oxide-aluminum vaccine against anthrax at the Kaluzhski bio-plant established that the vaccine possessed a high immunology when tested on guinea pigs. Thus, in 1954, 1955 and 1956, six series of vaccine were produced and subjected to tests. They were all released and the first lot proved a survival of 95.6% of vaccinated guinea pigs with a loss of all control animals. The vaccine is prepared with a content of 40-45 mln. of virulent spores in 1 ml.

For the testing of this vaccine, in order to determine its harmlessness during the spring injection period, a vaccination of sheep in the Georgian SSR was performed in April 1953. A total of 621 sheep were inoculated and in May, the same farm vaccinated another 380 sheep. The vaccinations were made with the hydro-oxide-aluminum series No. 13, prepared on 7 March 1953; dose = 0.2 ml. There were no complications in spite of the fact that the sheep were vaccinated in April (when they were) in an undernourished (emaciated) condition.

Wide tests to determine its harmlessness were conducted in autumn. In October 1953, the collective farm in the name of Kirov of the Iashvilenski district of the Stavropole region, the commission, consisting of: N. A. Mikhailov, I. Y. Borishov and V. M. Lashkov, vaccinated 8,548 sheep, including 4,548 of young ones and 40 goats and kids. The vaccine was injected subcutaneously into the left rear leg of grown animals - (a dose of 0.3 ml) and the young ones - (0.2 ml). The vaccinations produced no complicated cases. The attained results of the vaccination, performed on a relatively large number of sheep, proved the harmlessness of the hydro-oxide-aluminum vaccine, when used during spring and autumn.

In order to test the duration and resistance of the immunology created by the vaccine in April 1953, the commission (N. G. Malenkov, A. V. Kachidze, N. Y. Zaidzashvili) vaccinated the young sheep (born in 1952) at the experimental farm "Gedabno", Georgian SSR, which had not been previously vaccinated against anthrax.

Two series of the vaccine were used: Series No. 12, produced on 7 March 1953 on a 30% glycerin solution with a spore concentration of 47.3 mln per 1 ml and Series No. 13, prepared simultaneously with the mentioned medium with the same washing and concentration, but with an added 20% content of glycerin and hydro-oxide aluminum. In October a part of the lambs (the young sheep were castrated during the summer) were injected with a virulent culture of anthrax. In order to test the injections for immunology, 5 bullocks (animals) (vaccinated on 12 April 1953, with the

vaccine, Series No. 12) were used; 3 injected with hydro-oxide-aluminum vaccine Series No. 13 on 12 April 1953; 4 sheep, vaccinated with hydro-oxide-aluminum vaccine Series No. 18 on 17 October 1953; and 3 control bullocks (animals) which had not been vaccinated previously.

The inoculation of all sheep was made with a spore containing virus of anthrax Series No. 14 (prepared 27 February 1953) - 1 ml in a solution 1:20; subcutaneously, into the inside part of the rear right leg.

The control inoculation produced the following results. All 3 bullocks inoculated with the vaccine No. 12, survived, manifesting an insignificant fever reaction. All 3 bullocks inoculated with No. 13 survived and did not react to the inoculation with anthrax virus. Of 4 sheep, vaccinated 7 days prior to inoculation, 3 survived. Of 5 not vaccinated bullocks, inoculated with the virus simultaneously with the vaccinated, 4 perished from anthrax after 2.5 - 3, 3-4 and 5.5 days after inoculation.

Thus it was ascertained that the vaccinated bullocks and sheep produced a high immunity against anthrax.

In October and November 1954 an experiment was made on sheep to test the duration and resistance of the immunity, created by the anti-anthrax hydro-oxide-aluminum vaccine. The experiment was made by the commission consisting of S. G. Zolotarev, A. V. Kachhidze, S. G. Arzian and C. A. Mantashvili. An inoculation was made on bullocks, which were vaccinated with the hydro-oxide-aluminum vaccine during autumn of 1953 (dose of 0.3 ml), as well as sheep, vaccinated 7-14 days prior to the inoculation with 0.3 ml.

The inoculation was made with the virus of Series No. 16 (prepared 17 April 1954) - dose 1 ml in a solution 1:300, subcutaneously. Of the 10 bullocks, subjected to inoculation, 3 perished after 12.5 months after vaccination. One of them with a delay of 6 days in comparison with the control animals, 7 bullocks survived, of which 4 did not react to the anthrax virus, and one had a single slight rise of temperature. Five (5) sheep, vaccinated 14 days prior to inoculation, survived without manifesting any reaction. All 5 sheep, vaccinated with a combination (vaccine and serum) 14 days prior to inoculation, survived manifesting a brief fever reaction. Of 4 sheep vaccinated 7 days prior to inoculation, one perished and the remainder did not react to the virus. Four (4) sheep vaccinated (without hydro-oxide-aluminum) 7 days prior to inoculation, survived without manifesting any reaction to the virus. Five (5) unvaccinated control sheep perished from anthrax: 3 after 2.5 days, 1 after 3 days and 1 - 6 days after the inoculation.

Thus, the detailed test proved that the hydro-oxide-aluminum vaccine against anthrax possessed qualities which quickly created (with sheep) a resistant and lengthy (not less than a year) immunity.

In the autumn of 1954 the hydro-oxide-aluminum vaccine against anthrax was widely tested.



Vaccinations were made by local veterinary workers on the collective farms of the Stavropol'ski and Krasnodarski regions and the Rostovski Oblast.

We issued to the supervising veterinarians general directions for the vaccination. The hydro-oxide-aluminum vaccine against anthrax Series No. 2, 3 and 4 (produced by the Kaluzhski bio-plant in June 1954) was utilized.

Animals over 1 year of age received the vaccine subcutaneously in the following dosages: the large horned cattle - 1 ml; horses - 0.75; pigs - 0.5 ml; sheep 0.3 ml; and goats - 0.2 ml; young cattle, 3 months - 1 year; large horned cattle and horses - 0.1 - 0.5; pigs 0.3 ml; sheep 0.1 - 0.2 ml; and kids 0.1 ml.

The analysis of data received from local ones, where the vaccine had been used, only showed four complications among the animals: 5 sheep (0.0011%), 1 young bull (0.00076%), 1 foal (0.0034%) and 14 goats (0.23%).

The hydro-oxide-aluminum vaccine was used during spring and autumn of 1955. In accordance with the scrutinized material received from the Kaluzhski Oblast, 14,632 animals were vaccinated (in spring); this consisted of 306 horses, 4958 heads of large horned cattle, 8978 sheep, 1529 goats and 340 pigs.

Although some of the animals were fed inadequately, there were no complications and/or losses. Even among the vaccinated goats no complications were recorded.

Springtime vaccinations were made in districts of the Stavropole and Krasnodarsk regions, where (a total of) 8336 animals were vaccinated. The vaccinations manifested no complications. Thus, in accordance with the data received in spring of 1955, 24,934 animals were vaccinated.

Primary vaccinations of animals, with hydro-oxide-aluminum vaccine against anthrax, were given in the Rostov, Mursh, Orlovsk, Bryansk and Kaluzhski Oblasts during autumn of 1955, of the Stavropole and Krasnodarsk regions.

In accordance with files received, 2,137,034 animals were vaccinated; this represented 640,048 head of large horned cattle, 1,295,823 sheep, 95,803 horses, 37,170 pigs and 22,188 goats. This figure included 180,845 young large horned cattle, 457,300 sheep, 20,875 colts, 12,634 pigs and 1,939 kids.

During the analysis of the material, it was established that the reaction of the animals to the vaccine was insignificant with a few slight exceptions. With horses and large horned cattle it manifested itself by a few swellings, which occurred on the second day (2 x 3, 3 x 4 and less frequently 3 x 7 cm at the places of vaccination). After the 3-4th day the swellings decreased and became narrower and with a number of animals they dissolved completely. Sheep and goats also manifested swellings of 1 x 2, 2 x 3 cm on the second day, at the place of vaccination. These became limited after 3-4 days, and with some animals they dissolved completely

The animal temperature, following the vaccination, rose - 0.3-0.7-1.0° and in rare instances - 1.3-1.5°. It remained at this level for 1-3 days before dropping back to normal.

There were complications in two instances (16 head of large horned cattle, a loss of 8 young ones (0.00171) among sheep, complications occurred in 13 cases, with 397 animals. On 11 farms, 136 young animals perished, (0.011). Here it is necessary to mention that all cases of complications and loss of animals were caused by different reasons, as explained by the veterinarians. Cold and rainy weather, poor nutrition, inadequate care (maintenance) of the animals after the vaccination, etc.

Among the 95,883 horses there were no complications and no losses, though the work-horses were not excused from their duties. There were no complications and losses among inoculated pigs (57,170) and goats (21,148). Graphically, in evaluating the hydro-oxide-aluminum vaccine against anthrax, it was harmless for goats, who are particularly sensitive to anthrax injections.

The large horned cattle in the age group of 6 months to 3 years, in most cases, were vaccinated with the anti-anthrax hydro-oxide-aluminum vaccine simultaneously with an injection of formal vaccine against *sphaera carboles* (both sides of the neck). These vaccinations of thousands of large horned cattle were carried out without complications.

Thus- during 1954 and 1955 2,785,531 animals were vaccinated with hydro-oxide-aluminum anti-anthrax vaccine: of 803,956 heads of large horned cattle, 9 animals perished (0.0011%); of 127,722 horses, 1 horse (0.00078%); of 1,751,486 sheep, 141 (0.008%); of 28,529 goats, 14 (0.048%) and of 68,920 pigs - none.

As a result of careful computations and analyses of vaccinations, carried out during 1954-1955, it was established that the hydro-oxide-aluminum vaccine provided a resisting and durable immunity against anthrax. This was confirmed by the fact that in the numerous places where vaccinations were carried out, no cases of animal anthrax occurred.

In comparison with other anti-anthrax vaccines, the noted harmlessness of hydro-oxide-aluminum was evident, as it caused considerably less complications and losses than, for instance, Tsoukovski's second vaccine and CTN (BTI).

During 1956, approximately ten animals were vaccinated with hydro-oxide-aluminum. No complications were recorded. It is possible that there were insignificant complications, however, they must have been too negligible to be reported by the farmhands.

However, it must be also noted that in conducting a few experiments in respect to a comparative study of the reaction of the milk productivity of cows, the hydro-oxide-aluminum vaccine appeared less reactive than Tsoukovski's second vaccine and CTN (BTI). The vaccinated cows decreased their yield of milk considerably less during the period of temperature reaction of the organism.